Phenotypic robustness can increase phenotypic variability after nongenetic perturbations in gene regulatory circuits

Espinosa-Soto, C ; Martin, O C ; Wagner, A

Abstract: Nongenetic perturbations, such as environmental change or developmental noise, can induce novel phenotypes. If an induced phenotype appears recurrently and confers a fitness advantage, selection may promote its genetic stabilization. Nongenetic perturbations can thus initiate evolutionary innovation. Genetic variation that is not usually phenotypically visible may play an important role in this process. Populations under stabilizing selection on a phenotype that is robust to mutations can accumulate such variation. After nongenetic perturbations, this variation can produce new phenotypes. We here study the relationship between a phenotype's mutational robustness and a population's potential to generate novel phenotypic variation. To this end, we use a well-studied model of transcriptional regulation circuits that are important in many evolutionary innovations. We find that phenotypic robustness promotes phenotypic variability in response to nongenetic perturbations, but not in response to mutation. Our work suggests that nongenetic perturbations may initiate innovation more frequently in mutationally robust gene expression traits.

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Supporting information for: Phenotypic robustness can increase phenotypic variability after non-genetic perturbations in gene regulatory circuits

Carlos Espinosa-Soto Olivier C. Martin Andreas Wagner

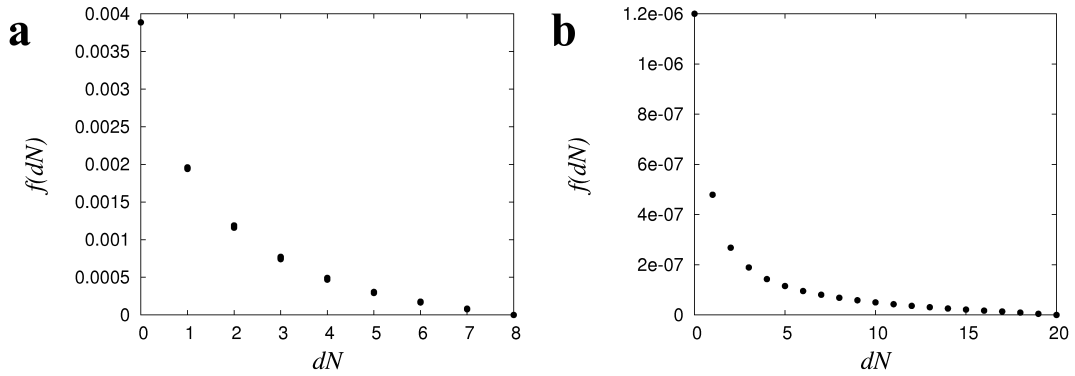

Fig. S1. The fraction of genotypes ($f(dN)$) that produce a phenotype decreases with dN. dN represents the number of gene activity differences between a predetermined initial condition, s_0, and the phenotype s_∞. To generate the data in this figure we constructed random genotypes with a specific number of genes N and with a specific number of regulatory interactions m. (a) $N = 8; m = 23$. Sampling included 2×10^7 genotypes. Spearman's $\rho = -0.978$; $p < 2.2 \times 10^{-16}$. Each point in the plot represents a specific phenotype. (b) $N = 20; m = 85$. Sampling included 10^8 genotypes. Spearman's $\rho = -1$; $p < 1.98 \times 10^{-6}$. Each point in the plot stands for the frequency of obtaining phenotypes with a given d, divided by the number of possible phenotypes with this distance d. All the phenotypes with equal d are equally frequent (Ciliberti, Martin & Wagner, 2007, PLoS Comput Biol, 3:e15). The number of phenotypes at a distance d from the fixed initial state s_0 is given by the binomial coefficient $\mathbf{C}_k^N$, where $k = dN$ stands for the number of gene expression differences between s_0 and s_∞.

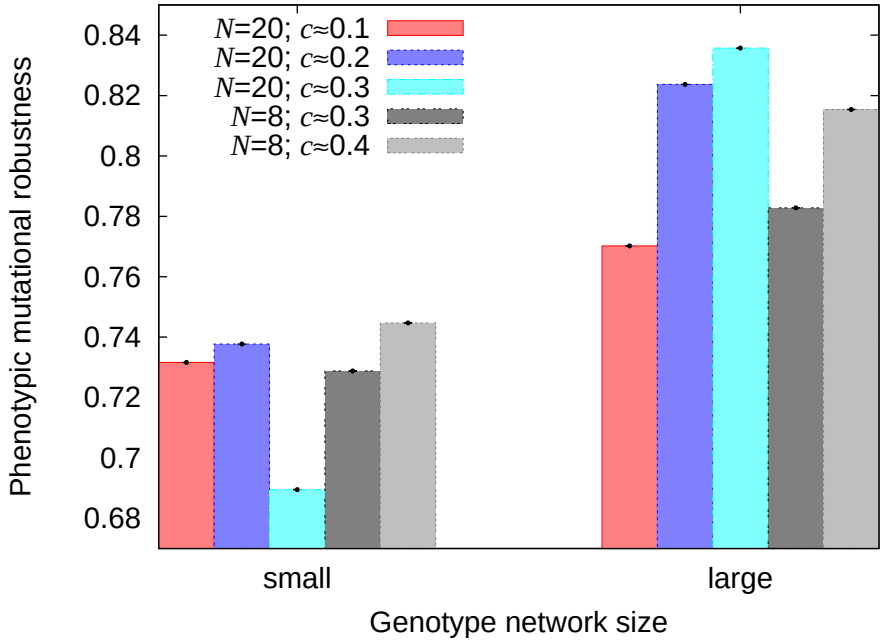

Fig. S2. Phenotypic mutational robustness increases with genotype network size. For the purpose of this figure, small genotype network size corresponds to $d = 0.5$. Large genotype network size corresponds to $d = 0.125$ when $N = 8$, and to $d = 0.1$ when $N = 20$. Hereafter we will refer to phenotypic mutational robustness as "phenotypic robustness". We sampled 10^6 genotypes in each genotype network. The length of bars represents one standard error.

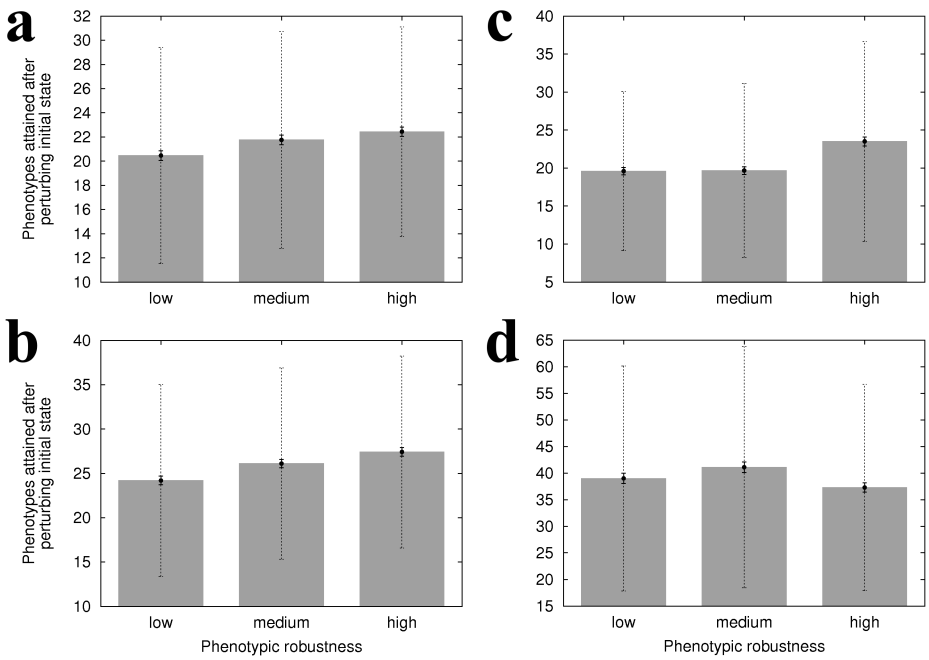

Fig. S3. High phenotypic robustness facilitates phenotypic variability in response to gene expression noise in s_0. Each plot shows mean values for 500 independent simulations at each level of phenotypic robustness. The length of solid error bars denotes one standard error. The length of dashed bars indicates one standard deviation. (a) $N = 8$; $c \approx 0.4$. (b) $N = 8$; $c \approx 0.3$. (c) $N = 20$; $c \approx 0.3$. (d) $N = 20$; $c \approx 0.1$. In this and all other figures, low phenotypic robustness corresponds to $d = 0.5$, medium robustness corresponds to $d = 0.25$; high robustness corresponds to $d = 0.125$ when $N = 8$, and to $d = 0.1$ when $N = 20$.

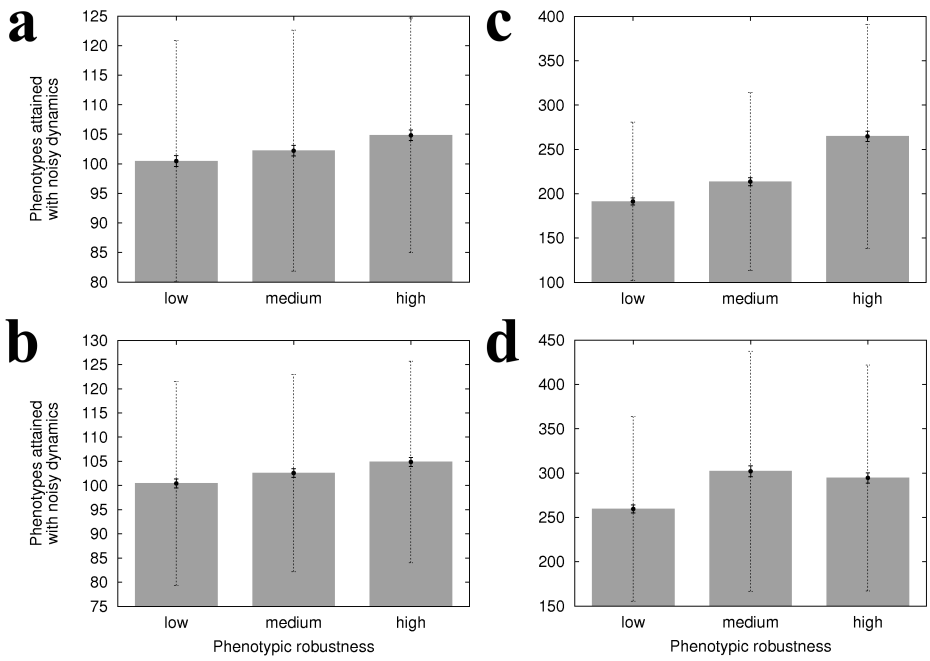

Fig. S4. High phenotypic robustness facilitates phenotypic variability in response to noisy gene expression dynamics. Each plot shows mean values for 500 independent simulations at each level of phenotypic robustness. The length of solid error bars denotes one standard error. The length of dashed bars indicates one standard deviation. (a) $N = 8$; $c \approx 0.4$. (b) $N = 8$; $c \approx 0.3$. (c) $N = 20$; $c \approx 0.3$. (d) $N = 20$; $c \approx 0.1$.

Table S1. The mean and maximum genetic distance within a population increases with phenotypic robustness according to a Mann-Whitney U test.

Genotype network			Mean genetic		Maximum genetic	
N	c	d	distance ±SE	p-value	distance ±SE	p-value
20	0.1	0.1	0.557±0.003	0.0059	0.874±0.003	9.65×10^{-5}
		0.5	0.545±0.003		0.854±0.003	
	0.2	0.1	0.434±0.003	3.59×10^{-5}	0.739±0.005	0.0056
		0.5	0.415±0.003		0.719±0.005	
	0.3	0.1	0.349±0.003	0.002	0.622±0.006	0.0056
		0.5	0.334±0.003		0.601±0.005	
8	0.3	0.125	0.697±0.002	4.17×10^{-14}	0.97±0.001	1.74×10^{-14}
		0.5	0.675±0.002		0.957±0.001	
	0.4	0.125	0.61 ±0.003	4.97×10^{-7}	0.901±0.002	5.22×10^{-10}
		0.5	0.593±0.002		0.886±0.002	

Mean and maximum genetic distance values are averaged across 500 populations for each combination of N, c and d. Each population comprises 200 gene circuits. Values of $d = 0.5$ correspond to low phenotypic robustness, and values of $d = 0.1$ or $d = 0.125$ correspond to high phenotypic robustness.

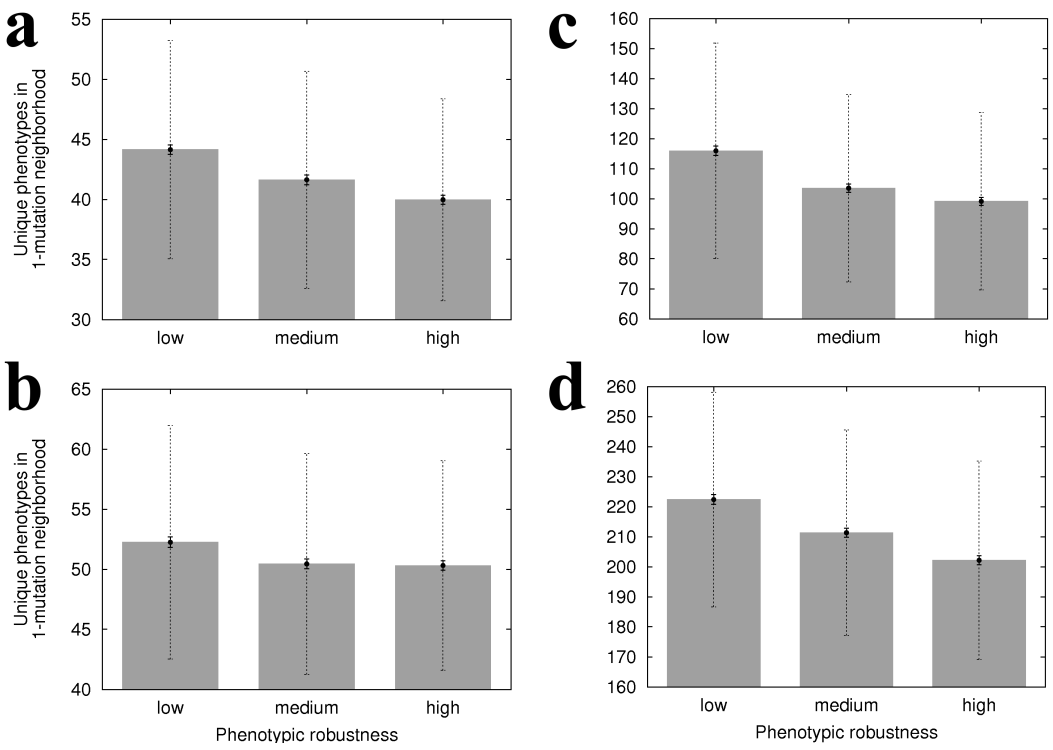

Fig. S5. High phenotypic robustness does not facilitate phenotypic variability in response to mutations without preceding environmental change. Each plot shows mean values for 500 independent simulations at each level of phenotypic robustness. The length of solid error bars denotes one standard error. The length of dashed bars indicates one standard deviation. (a) $N = 8$; $c \approx 0.4$. (b) $N = 8$; $c \approx 0.3$. (c) $N = 20$; $c \approx 0.3$. (d) $N = 20$; $c \approx 0.1$.

Table S2. Mean number of new phenotypes accessible from individual genotypes in populations evolving in different genotype networks.

Genotype network			Mean number of new phenotypes ± SE		
N	c	d	Mutation	Perturbing s_0	Noisy dynamics
20	0.1	0.1	6.5 ± 0.04	1.01 ± 0.03	6.97 ± 0.11
		0.5	7.3 ± 0.04	1.17 ± 0.03	6.53 ± 0.08
	0.2	0.1	5.59 ± 0.04	0.86 ± 0.02	7.24 ± 0.11
		0.5	6.46 ± 0.05	0.88 ± 0.02	6.66 ± 0.08
	0.3	0.1	4.33 ± 0.04	0.77 ± 0.02	7.34 ± 0.12
		0.5	5.32 ± 0.05	0.91 ± 0.01	6.42 ± 0.09
8	0.3	0.125	2.17 ± 0.02	0.97 ± 0.02	4.99 ± 0.04
		0.5	2.55 ± 0.02	1.05 ± 0.02	4.93 ± 0.04
	0.4	0.125	1.84 ± 0.02	0.81 ± 0.02	5.02 ± 0.05
		0.5	2.33 ± 0.02	0.95 ± 0.01	5.07 ± 0.05

For each genotype network we evolved 500 independent populations. Each population comprises 200 gene circuits. For each population evolved under stabilizing selection on an optimal gene activity phenotype s_∞^{opt} (see Methods in the main text), we counted the number of new phenotypes (induced by mutation or non-genetic perturbations) that are within reach of each genotype in the population.

Table S3. Relative phenotypic variability of genotypes in large genotype networks. We define this number as the number of new phenotypes that noise and mutation can produce per genotype in populations on a large genotype network for each new phenotype produced by the same kind of perturbation, but for populations on a smaller genotype network. This ratio is always greater for noise than for mutations, with one exception[a]. Numbers on this table based on data in Table S2.

Genotype network		Relative phenotypic variability of genotypes in large genotype networks.		
N	c	Mutation	Perturbing s_0	Noisy dynamics
20	0.1	0.891	0.859[a]	1.068
	0.2	0.865	0.972	1.088
	0.3	0.815	0.846	1.143
8	0.3	0.85	0.917	1.011
	0.4	0.79	0.846	0.991

[a] The only exception occurs for very low interaction densities ($N = 20; c \approx 0.1$; and perturbations in s_0), where in earlier analyses, populations had also not shown higher variability when evolving on large genotype networks (Fig. S3d).

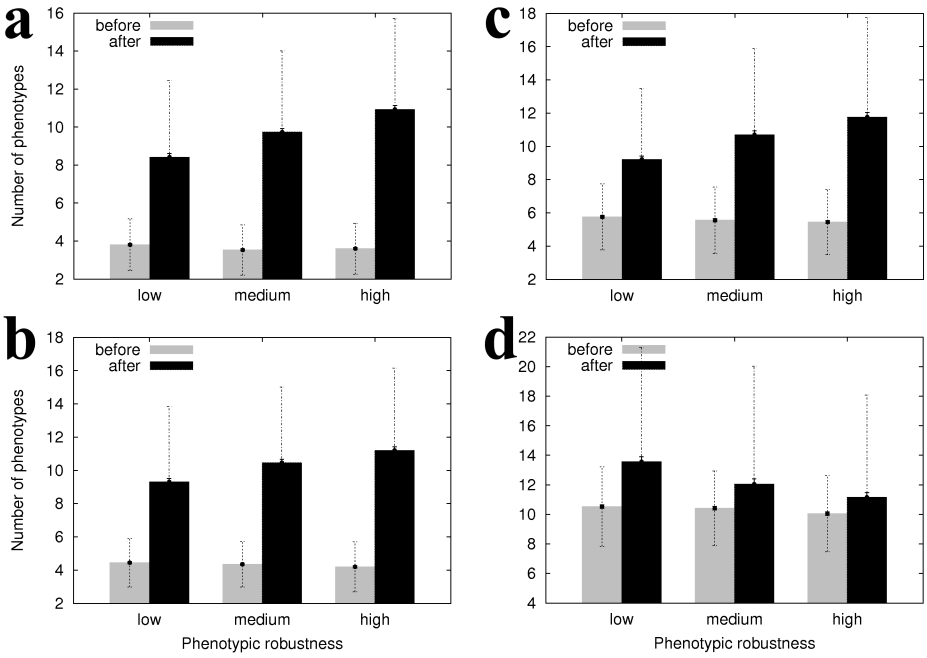

Fig. S6. High phenotypic robustness increases phenotypic diversity in populations of gene circuits after environmental change. The number of different phenotypes that populations display increases after shifting s_0. Such an increase is greater for populations with more robust phenotypes. Each plot shows mean values for 500 independent populations at each level of phenotypic robustness. The length of solid error bars denotes one standard error. The length of dashed bars indicates one standard deviation. (a) $N = 8$; $c \approx 0.4$. (b) $N = 8$; $c \approx 0.3$. (c) $N = 20$; $c \approx 0.3$. (d) $N = 20$; $c \approx 0.1$.

Table S4. The increase in the number of different phenotypes after changing s_0 is larger in circuits with robust phenotypes according to a Mann-Whitney U test.

Genotype network		P-value[a]
N	c	
20	0.2	7.8×10^{-5}
	0.3	1.49×10^{-18}
8	0.3	5.91×10^{-14}
	0.4	3.24×10^{-24}

[a]The comparison is performed between populations of circuits with $d = 0.1$ and $d = 0.5$ when $N = 20$, and with $d = 0.125$ and $d = 0.5$ when $N = 8$.

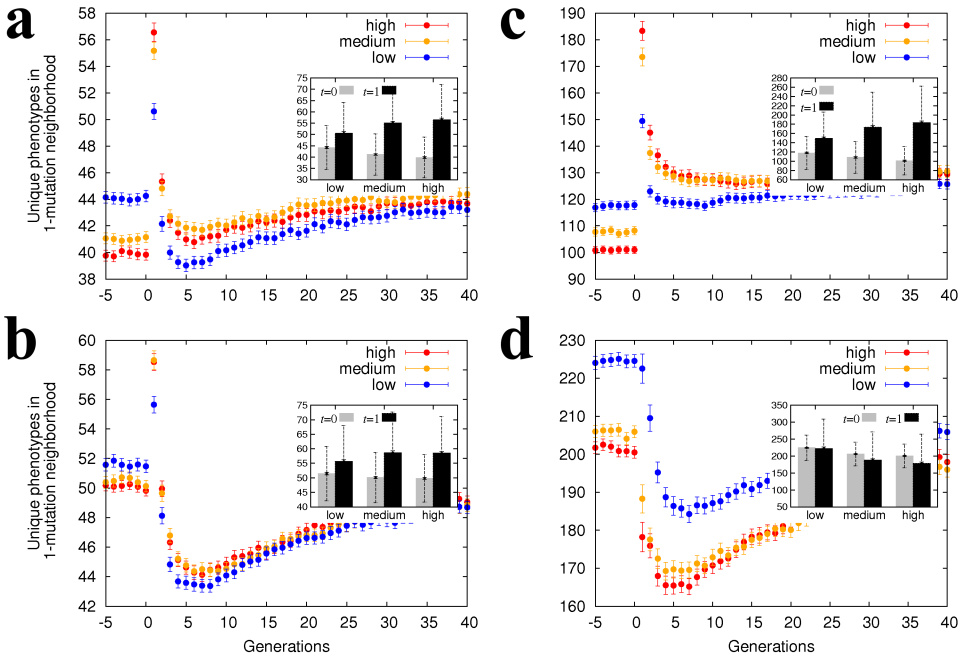

Fig. S7. High phenotypic robustness allows mutational access to more phenotypes after an alteration of the environment's inducing role. Each plot shows mean values of phenotypic variability for 500 independent simulations at each level of phenotypic robustness. We show in the insets the number of mutation-accessible phenotypes immediately before ($t = 0$) and immediately after ($t = 1$) replacement of s_0. The length of solid error bars denotes one standard error. The length of dashed bars (in the insets) indicates one standard deviation. (a) $N = 8$; $c \approx 0.4$. (b) $N = 8$; $c \approx 0.3$. (c) $N = 20$; $c \approx 0.3$. (d) $N = 20$; $c \approx 0.1$.

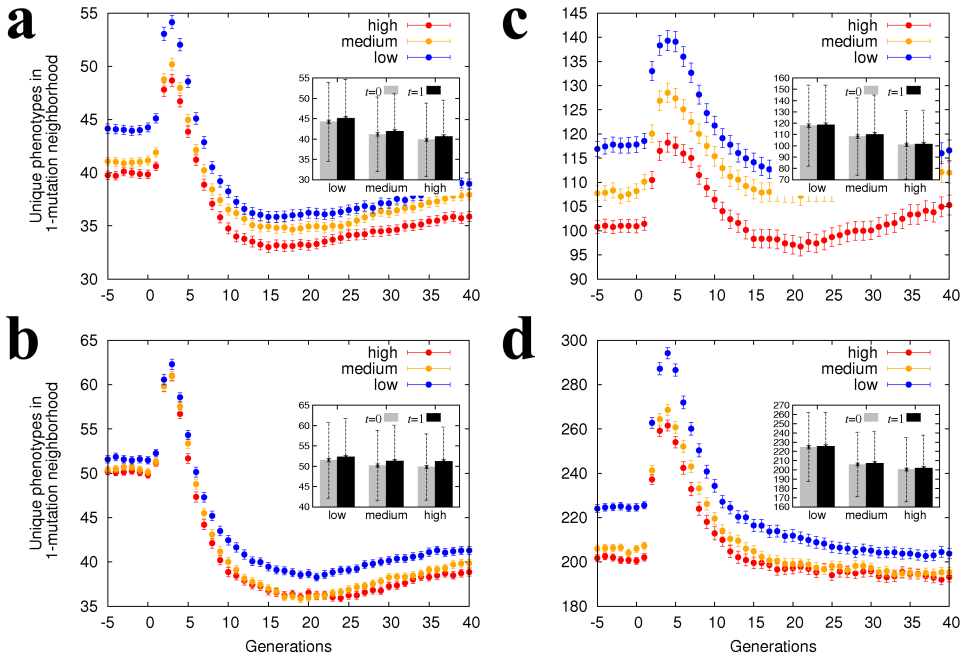

Fig. S8. High phenotypic robustness does not facilitate mutational access to more phenotypes after an alteration of the environment's evaluating role. Change of the evaluating role is implemented as a change in s_{∞}^{opt}. Each plot shows mean values of phenotypic variability for 500 independent simulations for each level of phenotypic robustness. We show in the insets the number of mutation-accessible phenotypes immediately before ($t = 0$) and immediately after ($t = 1$) replacement of s_{∞}^{opt}. The length of solid error bars denotes one standard error. The length of dashed bars (in the insets) indicates one standard deviation. (a) $N = 8$; $c \approx 0.4$. (b) $N = 8$; $c \approx 0.3$. (c) $N = 20$; $c \approx 0.3$. (d) $N = 20$; $c \approx 0.1$.

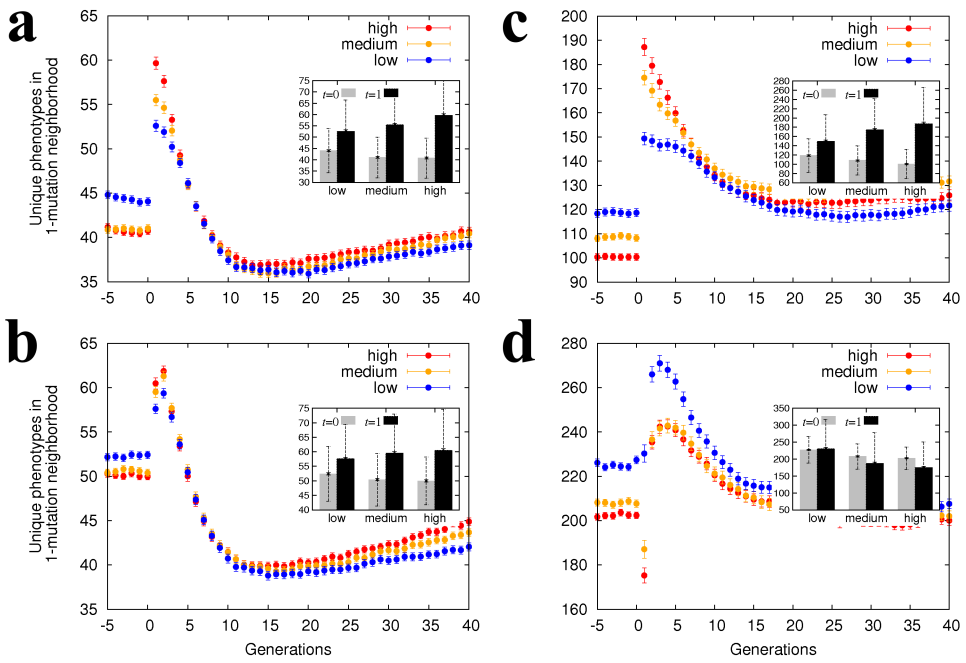

Fig. S9. High phenotypic robustness allows mutational access to more phenotypes after an alteration of both the inducing and evaluating role of the environment. Each plot shows mean values of phenotypic variability for 500 independent simulations for each level of phenotypic robustness. We show in the insets the number of mutation-accessible phenotypes immediately before ($t = 0$) and immediately after ($t = 1$) replacement of s_{∞}^{opt} and s_0. The length of solid error bars denotes one standard error. The length of dashed bars (in the insets) indicates one standard deviation. (a) $N = 8$; $c \approx 0.4$. (b) $N = 8$; $c \approx 0.3$. (c) $N = 20$; $c \approx 0.3$. (d) $N = 20$; $c \approx 0.1$.

Table S5. The increase in the number of accessible new phenotypes is larger in circuits with robust phenotypes, according to a Mann-Whitney U test.

Genotype network		P-value[a]
N	c	
20	0.2	1.78×10^{-21}
	0.3	5.41×10^{-71}
8	0.3	2.38×10^{-16}
	0.4	1.3×10^{-45}

[a]The comparison is performed between populations of circuits with $d = 0.1$ and $d = 0.5$ when $N = 20$, and with $d = 0.125$ and $d = 0.5$ when $N = 8$.

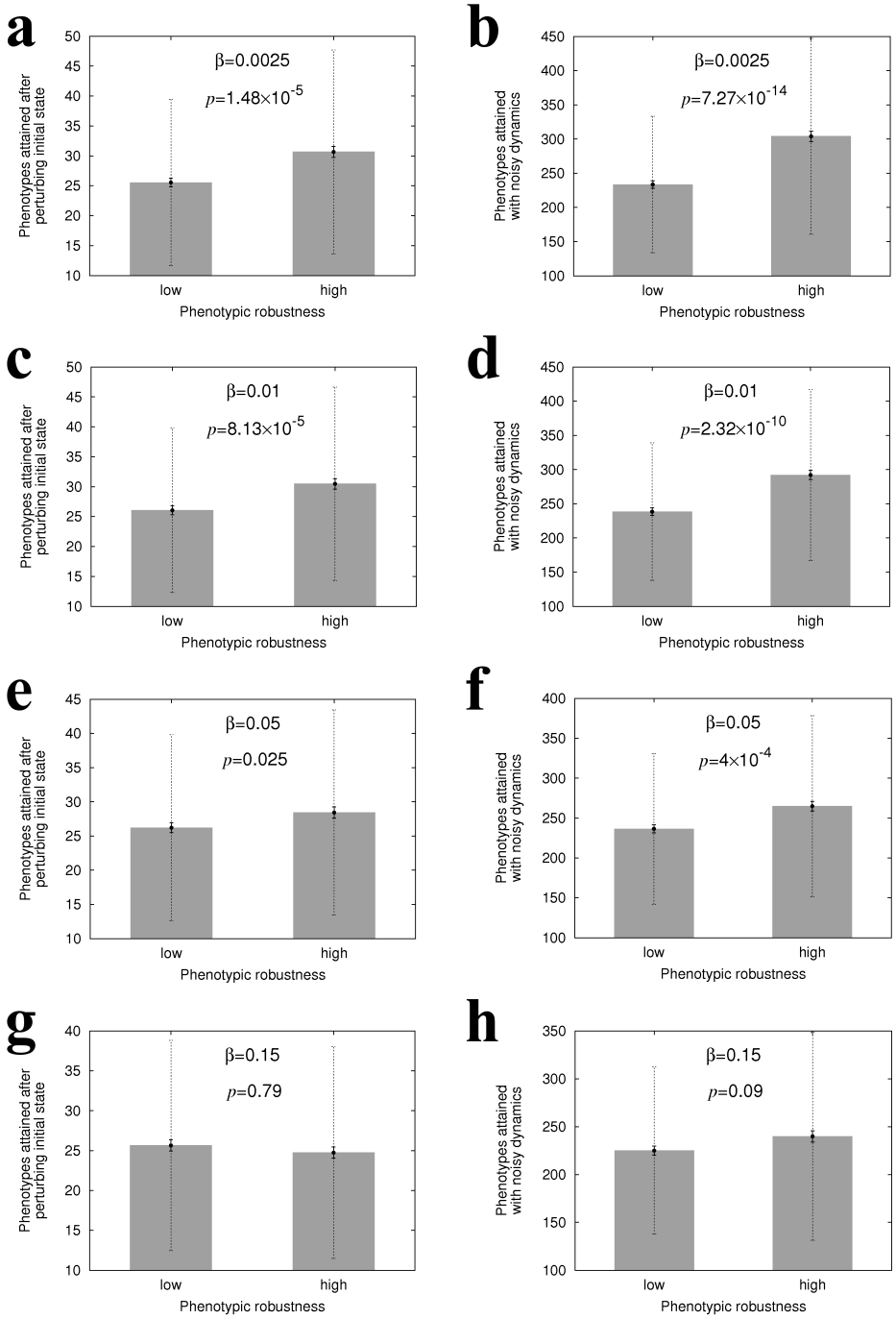

Fig. S10. Phenotypic variability in response to gene expression noise increases with phenotypic robustness in populations evolved under stabilizing selection and recurrent non-genetic perturbations. The positive effect of phenotypic robustness is statistically significant, according to a Mann-Whitney U test. This positive effect exists as long as the probability β of a circuit undergoing non-genetic perturbations throughout the period of stabilizing selection is not too high. The panels show phenotypic variability either after perturbations in the activity of single genes in the initial condition (a,c,e,g) or after perturbations of a circuit's gene expression dynamics (b,d,f,h). Each plot shows mean values for 350 independent simulations at each level of phenotypic robustness. The length of solid error bars denotes one standard error. The length of dashed bars indicates one standard deviation. In all panels $N = 20$ and $c \approx 0.2$.

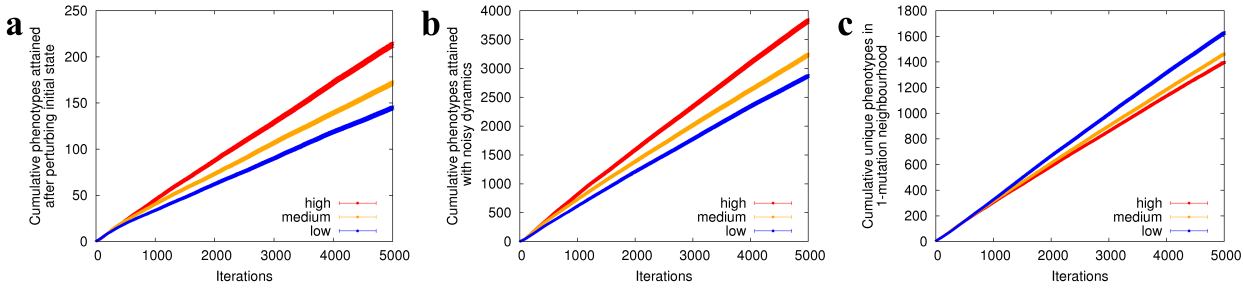

Fig. S11. Cumulative number of new phenotypes that a single gene circuit encounters after iterative rounds of mutation that preserve its phenotype. Each plot shows mean values for 500 independent simulations at each level of phenotypic robustness. (a) New phenotypes accessible through gene expression noise in s_0. (b) New phenotypes accessible through noisy gene expression dynamics. (c) New phenotypes accesible through mutation. $N = 20$; $c \approx 0.2$.